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BOLU ABANT İZZET BAYSAL UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
Department of Chemistry

BIOCHEMICAL ALTERATIONS IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) AFTER ACUTE EXPOSURE TO SILDENAFIL

MASTER OF SCIENCE

AYSU PELİT

ACADEMIC SUPERVISOR
PROF. DR. AZRA BOZCAARMUTLU BÜKEN
ACADEMIC CO-SUPERVISOR
ASSIST. PROF. DR. CANAN SAPMAZ

BOLU, JULY - 2024

APPROVAL OF THE THESIS

BIOCHEMICAL ALTERATIONS IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) AFTER ACUTE EXPOSURE TO SILDENAFIL submitted by **Aysu PELİT** and defended before the Examining Committe Members listed below in partial fulfillment of the requirements for the degree of **Master of Science** in **Department of Chemistry, Institute of Graduate Studies of Bolu Abant Izzet Baysal University in 22.07.2024** by

Examining Committee Members	**Signature**
Supervisor Prof. Dr. Azra BOZCAARMUTLU BÜKEN Bolu Abant İzzet Baysal University	.....
Member Prof. Dr. Özlem YILDIRIM Ankara University	.....
Member Assoc. Prof. Dr. Ercan Selçuk ÜNLÜ Bolu Abant İzzet Baysal University	.....

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Aysu PELİT

ABSTRACT

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MSC THESIS
AYSU PELİT
BOLU ABANT İZZET BAYSAL UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF CHEMISTRY
(SUPERVISOR: PROF. DR. AZRA BOZCAARMUTLU BÜKEN)
(CO-SUPERVISOR: ASSIST. PROF. DR. CANAN SAPMAZ)
BOLU, JULY 2024
xii + 43

Sildenafil (Viagra) is a drug used to treat erectile dysfunction in men. Aquatic organisms expose to this chemical due to the increased usage of it. However, its effect has not been well studied in fish. The aim of this study was to examine the effect of 2 different doses of sildenafil on biochemical analysis including cytochrome P450 (CYP) and antioxidant enzyme activities in rainbow trout (*Oncorhynchus mykiss*). The animals were treated with sildenafil for 96 hours. At the end of this period, fish were killed and liver tissues were taken. The impact of sildenafil was measured in microsomes and cytosols of liver tissues. The results of this study showed that sildenafil had no acute effect on CYP1A-associated ethoxyresorufin O-deethylase, CYP3A-associated erythromycin N-demethylase, glutathione S-transferase, catalase and glutathione reductase activities. Sildenafil did not significantly alter cytochrome P450 and antioxidant enzyme activities in rainbow trout at these doses and within this administration period.

KEYWORDS: Antioxidant enzyme activities, Cytochrome P450, Fish, Liver, Sildenafil

ÖZET

**GÖKKUŞAĞI ALABALIĞI'NDA (*ONCORHYNCHUS MYKISS*)
SİLDENAFİLE AKUT MARUZ KALMA SONRASI
BİYOKİMYASAL DEĞİŞİKLİKLER
YÜKSEK LİSANS TEZİ
AYSU PELİT
BOLU ABANT İZZET BAYSAL ÜNİVERSİTESİ
LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ
KİMYA BÖLÜMÜ
(TEZ DANIŞMANI: PROF. DR. AZRA BOZCAARMUTLU BÜKEN)
(İKİNCİ DANIŞMAN: DR. ÖĞR. ÜYESİ CANAN SAPMAZ)
BOLU, TEMMUZ - 2024
xii+ 43**

Sildenafil (Viagra), erkeklerde ereksiyon bozukluğunu tedavi etmek için kullanılan bir ilaçtır. Sucul organizmalar kullanımının artması nedeniyle bu kimyasala maruz kalmaktadır. Ancak etkisi balıklarda yeterince araştırılmamıştır. Bu çalışmanın amacı gökkuşağı alabalığında (*Oncorhynchus mykiss*) 2 farklı dozda sildenafilin sitokrom P450 sistemi (CYP) ve antioksidan enzim aktivitelerini içeren biyokimyasal analizler üzerine etkisini incelemektir. Hayvanlara 96 saat boyunca sildenafil uygulanmıştır. Bu sürenin sonunda balıklar öldürülerek karaciğer dokuları alınmıştır. Sildenafilin etkisi karaciğer dokularının mikrozomlarında ve sitozollerinde ölçülmüştür. Bu çalışmanın sonuçları sildenafilin CYP1A ile ilişkili etoksiresorufin O-deetilaz, CYP3A ile ilişkili eritromisin N-demetilaz, glutatyon S-transferaz, katalaz ve glutatyon redüktaz enzim aktiviteleri üzerinde akut bir etkisinin olmadığını göstermiştir. Sildenafil bu dozlarda ve bu uygulama süresi içerisinde gökkuşağı alabalığında sitokrom P450 ve antioksidan enzim aktivitelerini anlamlı ölçüde değiştirmemiştir.

ANAHTAR KELİMELER: Antioksidan enzim aktiviteleri, Sitokrom P450, Balık, Karaciğer, Sildenafil

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LIST OF ABBREVIATIONS AND SYMBOLS

CAT : Catalase

CDNB: 1-chloro-2,4-dinitrobenzene

CYP : Cytochrome P450

ERND : Erythromycin N-demethylase

EROD : 7-ethoxyresorufin O-deethylase

FAD : Flavin adenine dinucleotide

GR : Glutathione reductase

GSH : Reduced glutathione

GSSG : Oxidized glutathione

GSTs : Glutathione S-transferases

KPi : Potassium phosphate buffer

min : Minute

$NADP^+$: β-nicotinamide adenine dinucleotide phosphate oxidized form

NADPH : Nicotinamide adenine dinucleotide phosphate reduced form

PAHs : Polycyclic aromatic hydrocarbons

PDE5 : Phosphodiesterase type 5

PCBs : Polychlorinated biphenyls

ROS : Reactive oxygen species

SEM : Standard error of mean

cGMP : Cyclic guanosine monophosphate

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1. INTRODUCTION

Environmental pollution began with the advent of humans. It is known that when *Homo sapiens* first made fire, the resulting smoke represented the earliest form of environmental pollution (1). The Industrial Revolution, coupled with population growth, caused rapid escalation in environmental pollution. As the population has grown, the rate of chemical usage has increased, and today, the use of chemicals is considered as a major reason of environmental pollution globally (2). Most waste products contaminate the environment, as well as atmospheric and marine systems. This increase in pollution in recent years has adversely affected all living organisms. Chemicals like insecticides, heavy metals, persistent organic pollutants (POPs) and hydrocarbons are a few examples of pollutants (2). These contaminants can lead to harmful health effects including cancer and diseases related with disruption of organs related with reproduction, biotransformation and excretion systems.

In addition to pesticides, heavy metals and the other persistent organic compounds, pharmaceutical chemicals are frequently detected in the environment. The presence of these chemicals has long been recognized as a significant concern in aquatic ecosystems. Pharmaceuticals have been increasingly detected in recent studies in aquatic environments with amounts of ng to μg/L quantities. This has primarily been linked to the shortcomings of wastewater treatment facilities (3) (Figure 1.1). The biologically active nature of these micropollutants renders them capable of inducing sub-lethal or chronic toxic effects, thereby giving rise to significant toxicological concerns (4). Sildenafil is also one of these pharmacological pollutants.

Figure 1.1. Wastewater treatment plant (5).

1.1 Sildenafil

Sildenafil (Viagra™, Pfizer, Inc.) improves penile erections in men with erectile dysfunction (ED). Erectile dysfunction (ED) poses a significant concern among males aged 40 years and above, with over half of individuals in this broad age range encountering varying degrees of ED. Therefore, the availability of orally administered effective treatment in the form of Viagra (sildenafil citrate) provides a significant therapeutic breakthrough (Figure 1.2) (6).

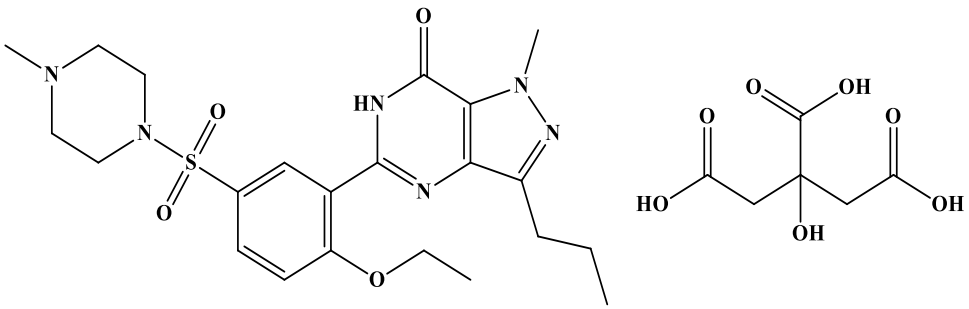

Figure 1.2. Sildenafil citrate structure.

Sildenafil is an inhibitor of phosphodiesterase type 5 (PDE5) enzyme. This enzyme is present in the smooth muscle of vascular system. It is specific to cyclic guanosine monophosphate (cGMP) (7). Sildenafil is also sold under the brand name Revatio. It is used to treat the patient suffering from pulmonary arterial

hypertension. In the treatment of this disease, it relaxes the blood vessels in the lungs. Sildenafil and other PDE5 inhibitors are used in the treatment of vasospasm, ischemia and ulcers observed in the patients suffering from secondary Raynaud's phenomenon (Figure 1.3) (8, 9).

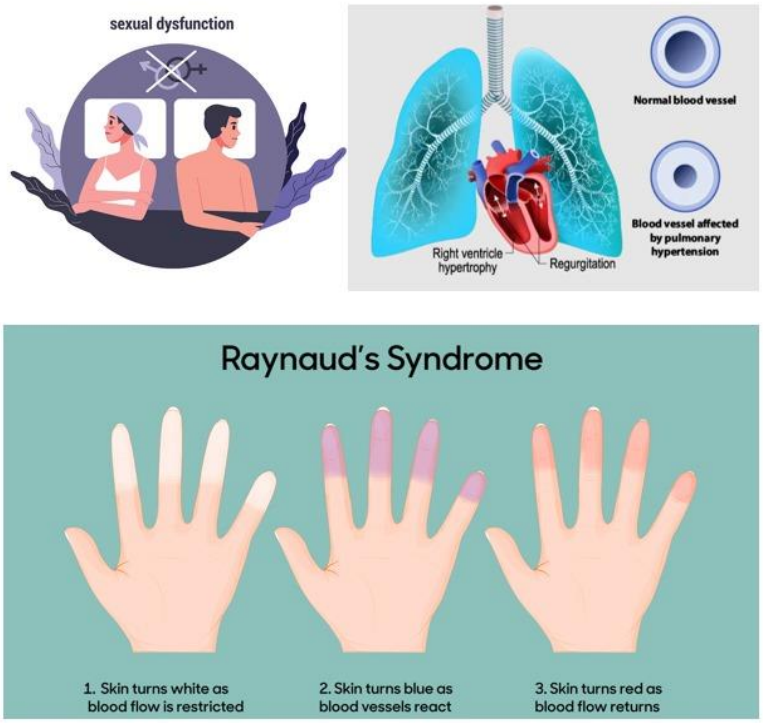

Figure 1.3. Therapeutic indications of sildenafil (10) (11) (12).

Sildenafil citrate is metabolized in the liver of humans. It is mainly metabolized by the enzyme cytochrome P450 3A4, and substances that inhibit this enzyme (such as macrolide antibiotics, antifungals, and cimetidine) could elevate sildenafil serum levels, resulting in intensified pharmacological and toxicological effects. Cytochrome P4503A enzyme has a key role in the conversion of sildenafil into active metabolites (Figure 1.4) (13). Metabolites produced because of sildenafil metabolism are typically excreted via urine and feces (14).

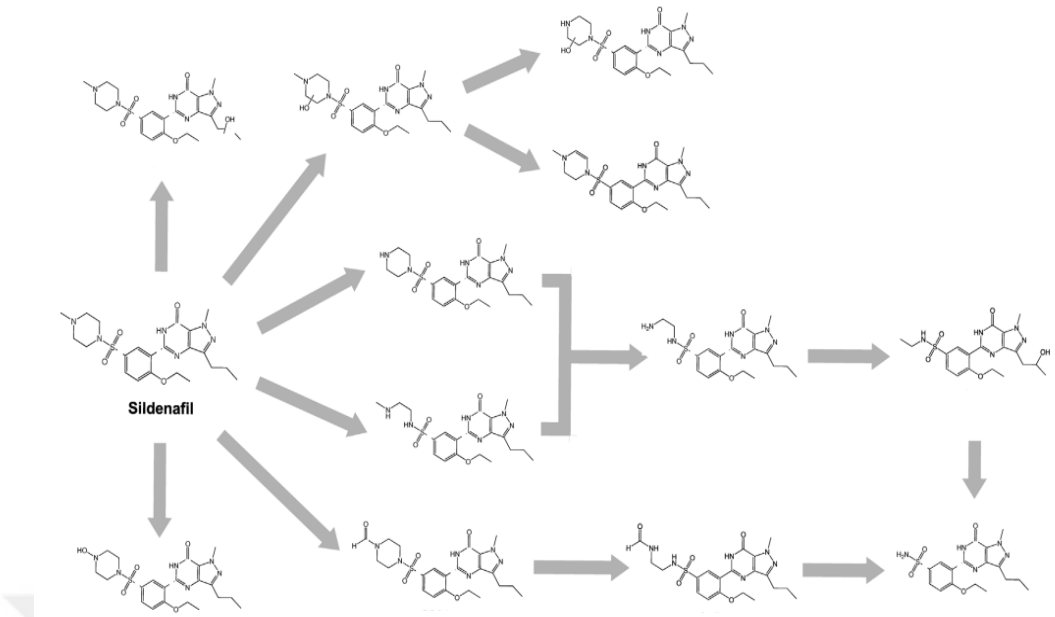

Figure 1.4. Sildenafil metabolites (13).

1.2 Biotransformation

The process by which a living organism alters the chemical structure of a drug within the body is termed drug metabolism or biotransformation (15). Chemical modifications of drugs, akin to the chemical transformations occurring in normal metabolic processes, do not occur spontaneously. All drug biotransformation are catalyzed reactions, occurring solely in the presence of enzymes. These enzymes, acting as protein catalysts, accelerate the process while seemingly undergoing no change themselves (16).

The liver serves as the primary organ for drug biotransformation reactions. Other organs involved in drug biotransformation include the lungs, skin, kidneys, and gastrointestinal tract (17). Drug biotransformation encompasses four primary types of metabolic reactions: oxidation, reduction, hydrolysis, and conjugation. Phase I (nonsynthetic) and Phase II (synthetic) reactions are the two groups into which these reactions fall. Phase II reactions include conjugation reactions, while Phase I has reactions namely hydrolysis, oxidation and reduction (Figure 1.5) (16, 17).

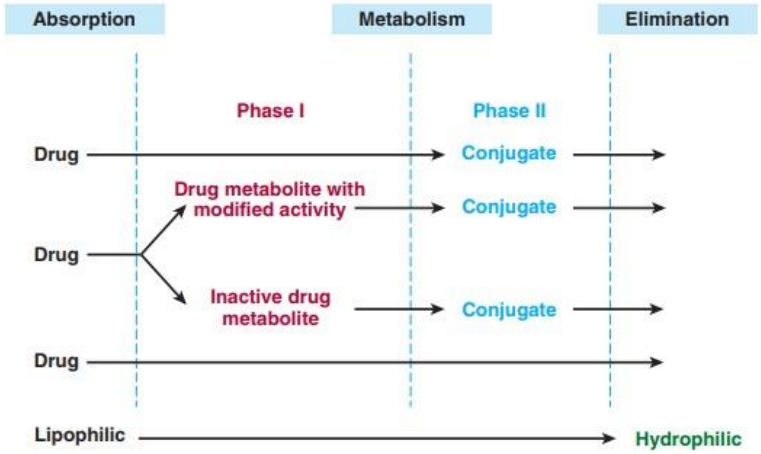

Figure 1.5. General biotransformation pathway (17).

Typically, these Phase I reactions merely mark the initial phase of biotransformation. Most drugs undergo a two-stage biotransformation process (18). The products resulting from Phase I reactions are generally not excreted from the body, despite often being less lipid-soluble than their parent drugs. The excretion of these products occurs solely after Phase II reaction. At the conclusion of Phase I, chemicals undergo conversion into forms that may be equally active, more active, less active, or toxic. At the culmination of Phase II reactions, drugs are transformed to inactive forms, and then excreted (17, 18).

1.2.1 Cytochrome P450

The cytochrome P450 (CYP) system encompasses a vast superfamily of heme proteins engaged in the oxidative metabolism of a multitude of lipophilic exogenous and endogenous substrates. The majority of Phase I reactions are facilitated by CYPs. These heme-containing proteins are predominantly located in the liver. The designation "450" denotes the peak wavelength value in nanometers at which light is absorbed by liver pigments containing heme proteins subsequent to their binding with carbon monoxide (CO) when reduced with sodium dithionite ($Na_2S_2O_4$) (Figure 1.6) (19).

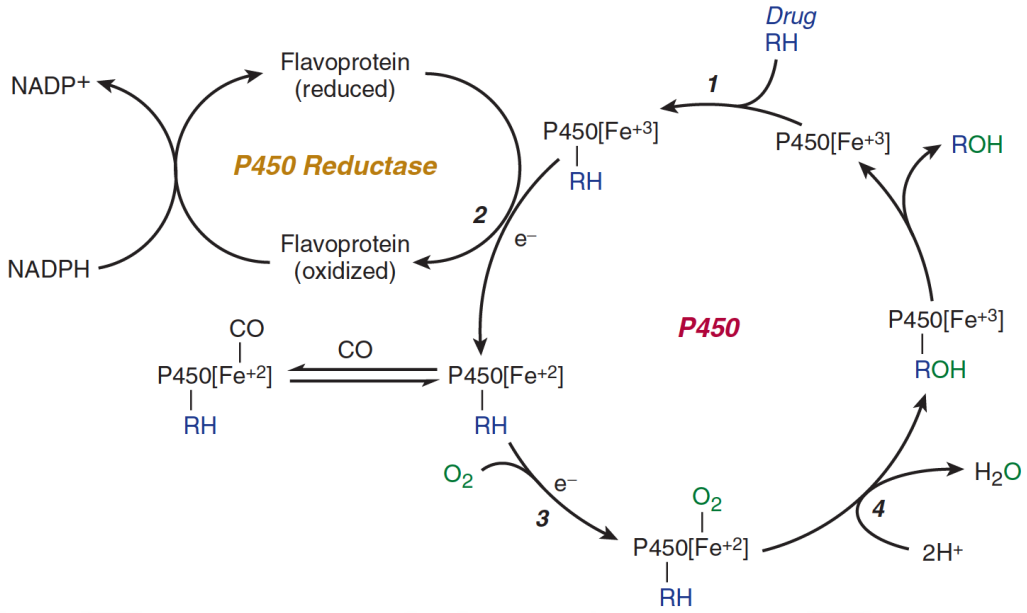

Figure 1.6. The cytochrome P450 reaction (17).

Cytochrome P450s are membrane-bound proteins. They are found in endoplasmic reticulum and mitochondria (20, 21). CYPs facilitate the metabolism of thousands of endogenous and exogenous chemicals. Exogenous substances encompass a range of compounds including drugs, alcohols, antioxidants, environmental pollutants, and various chemicals (20, 21). Numerous drugs have the ability to alter the activities of different CYPs, either by directly preventing the activity of specific CYPs through enzyme inhibition or by stimulating specific CYPs by increasing biosynthesis. CYP enzymes have very important roles in xenobiotic metabolism (21).

1.2.1.1 Cytochrome P450 System in Fish

Pesticides, heavy metals, and other contaminants present in water bodies are among the chemical stress factors to which fish are exposed. These pollutants can induce or inhibit cytochrome P450 enzymes, thereby affecting the physiological status and health of fish. Additionally, cytochrome P450 enzymes present in the liver of fish play a key role, particularly in detoxification processes. Research indicates that the biotransformation capacities of cytochrome P450 enzymes in fish can vary among species (22). These differences are associated with the habitats they inhabit, their feeding habits, and genetic structures. Therefore, the cytochrome P450 system is a significant research topic in fish ecology and toxicology, providing valuable insights into the health of aquatic ecosystems (22).

1.2.1.2 CYP1A Subfamily

The CYP1A subfamily stands out as pivotal in the bioactivation process of chemical carcinogens. Two distinct CYP1A genes have been identified in fish, CYP1A1 and CYP1A2. The CYP1A enzyme serves as a significant biomarker in fish, contributing substantially to the monitoring of pollutants within aquatic ecosystems (23, 24). CYP1A induction is generally followed by measuring 7-ethoxyresorufin O-deethylase (EROD) activity in liver microsomes (23). Measurement of EROD activity act as early warning sign for exposure to specific polycyclic aromatic hydrocarbons (PAHs) and closely related compounds in fish (24).

1.2.1.3 CYP3A Subfamily

CYP3A facilitates the catalysis of both endogenous substances such as bile acids and steroids, as well as exogenous molecules including pharmaceutical drugs (25). The significance of CYP3A in drug metabolism arises from hepatic first-pass metabolism of many drugs or as part of the metabolism terminating the effects of drugs. Due to their decisive role in the metabolism of substrates and drug interactions, CYP3A enzymes hold great importance in pharmacological and toxicological studies (17, 25). The assessment of CYP3A levels commonly involves quantifying erythromycin N-demethylase activity (ERND). CYP3A mediates the metabolic conversion of erythromycin to its N-demethylated metabolite along with the production of formaldehyde (26).

1.2.2 Glutathione S-Transferase

Prokaryotic and eukaryotic organisms have glutathione S-transferases (GSTs). They are part of phase II reactions. They catalyze the conjugation reactions of many xenobiotics with reduced form of GSH (27). In general, detoxification processes of chemicals are catalyzed by GSTs. Three different superfamilies compose the GST family: cytosolic, mitochondrial, and microsomal proteins (27, 28). By conjugating GSH with electrophilic sites found on a variety of substrates using a sulfhydryl group, GSTs enhance the solubility of these molecules in water (Figure 1.7) (29). These enzymes are primarily found in the liver (27). The synthetic substrate 1-chloro-2,4-dinitrobenzene (CDNB) serves as a collective molecule employed by all GSTs (27).

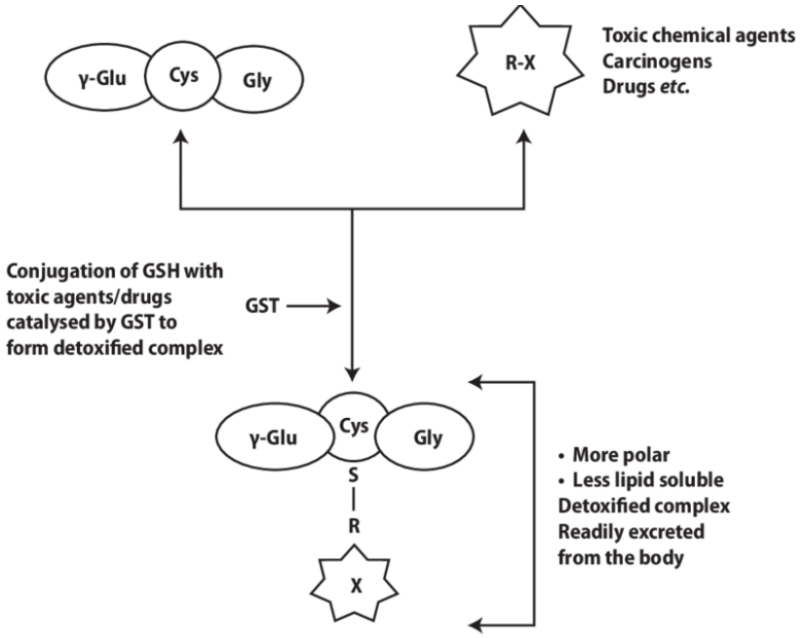

Figure 1.7. Glutathione- S transferase reaction (29).

1.3 Oxidative Stress and Antioxidant Systems

Oxidative stress is characterized by the imbalance among the reactive oxygen species (ROS) and the body's antioxidant defense system (Figure 1.8) (30). It may result in tissue damage.

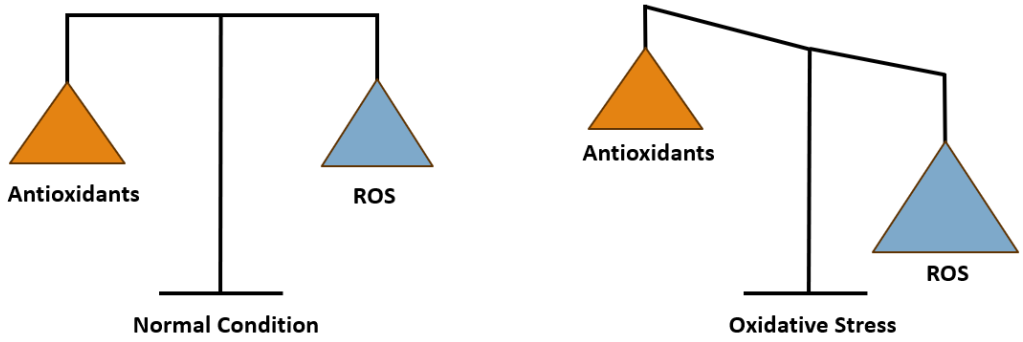

Figure 1.8. The relationship between oxidative stress and the antioxidant system (30).

Free radicals are highly reactive and unstable molecules generated naturally in the body as a byproduct of normal metabolic processes or through exposure to environmental toxic chemicals like tobacco smoke and ultraviolet radiation (31). Free radicals can either donate or accept an electron from other molecules, thus acting as either oxidizing agents or reducing agents (31). The major types of ROS

are hydroxyl radicals, hydrogen peroxide, and superoxide anions. ROS can interact with proteins and lipids, leading to detrimental effects within the cell (31). If antioxidant defenses are insufficient, various tissues may suffer from damage (32).

Living organisms protect themselves from the adverse impacts of free radicals via antioxidants. They are classified into as enzymatic and non-enzymatic antioxidants (33). Antioxidant enzymes catalyze the breakdown and elimination of free radicals. They facilitate the conversion of oxygen radicals to hydrogen peroxide (H_2O_2), which is subsequently metabolized into water (33, 34). Enzymatic antioxidants include superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase. Other antioxidants type is the non-enzymatic antioxidants act as scavengers for free radicals. They include vitamin C, vitamin E and glutathione (Figure 1.9) (33).

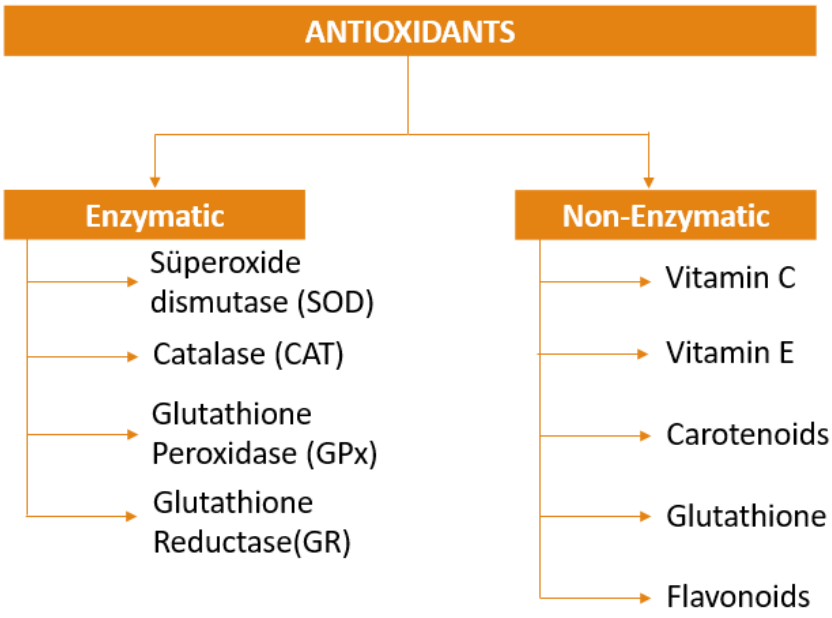

Figure 1.9. Classification of Antioxidants (33).

1.3.1 Catalase

Catalase (CAT) is a widely distributed antioxidant enzyme found in nearly all living tissues, functioning to degrade or convert hydrogen peroxide into water and molecular oxygen, with iron or manganese serving as cofactors in this process (Figure 1.10) (35). Catalase predominantly localized within peroxisomes (36). It is a tetrameric protein with four subunits (34, 36). It protects living organisms from the deleterious effect of hydrogen peroxide (36, 37). The decomposition of hydrogen peroxide occurs in two stages when catalase is present. In the initial step,

a single H_2O_2 molecule oxidizes heme to an oxyferryl species, removing one oxidation equivalent from both the iron and the porphyrin ring (Por), thus forming a porphyrin cation radical. In the second step, another hydrogen peroxide molecule reduces compound I, restoring the enzyme to its resting state and producing water and oxygen. Ultimately, compound I is further oxidized by a second H_2O_2 molecule, regenerating the resting enzyme, along with the formation of water and oxygen. Consequently, two hydrogen peroxide molecules are transformed into 2 water molecules and 1 oxygen molecule (Figure 1.10) (36,37).

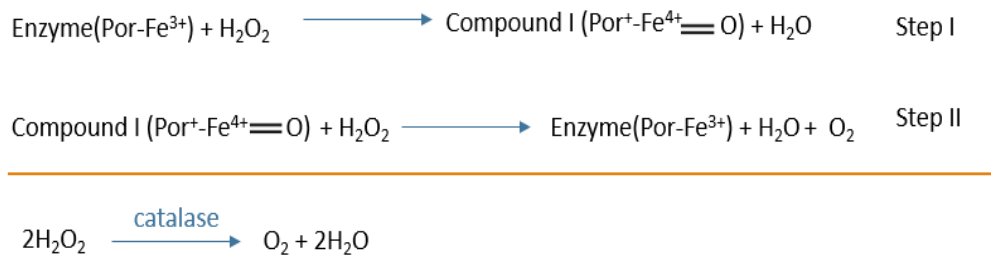

Figure 1.90. The decomposition reaction of hydrogen peroxide with catalase enzyme (35).

1.3.2 Glutathione Reductase

Glutathione reductase (GR) facilitates the conversion of glutathione disulfide (GSSG) to its sulfhydryl form, glutathione (GSH), a vital molecule for counteracting oxidative stress (38). It preserves the cellular reducing environment. GR operates as a dimeric disulfide oxidoreductase, employing a FAD prosthetic group along with NADPH to catalyze the reduction of one mole of GSSG to yield two moles of reduced GSH (38-40). Figure 1.11 shows the general reaction catalyzed by GR.

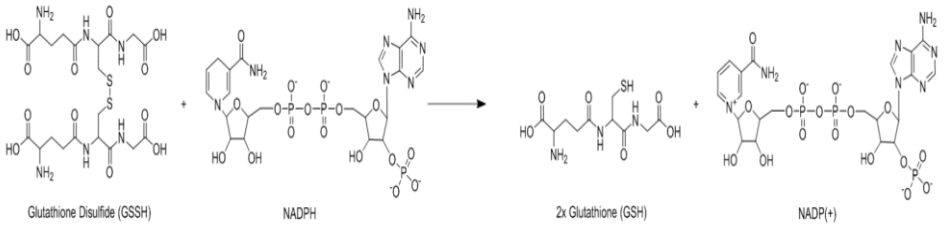

Figure 1.11. Glutathione Reductase-Mediated Reduction of Glutathione Disulfide (40).

L-glutamate and L-cysteine participate in the formation of γ-glutamylcysteine through the enzymatic action of γ-glutamylcysteine synthetase (39-41). This intermediate subsequently combines with glycine to produce glutathione in a reaction catalyzed by glutathione synthetase, consuming two molecules of ATP. The presence of Mg(II) or Mn(II) is crucial on the activity of γ-glutamylcysteine synthetase (39). The thiol group on the cysteine residue serves as the active functional moiety of glutathione. Upon oxidation, an intermolecular dipeptide bond forms, rendering the molecule non-functional. Glutathione reductase is responsible for restoring the reduced form of glutathione (Figure 1.12) (39-41).

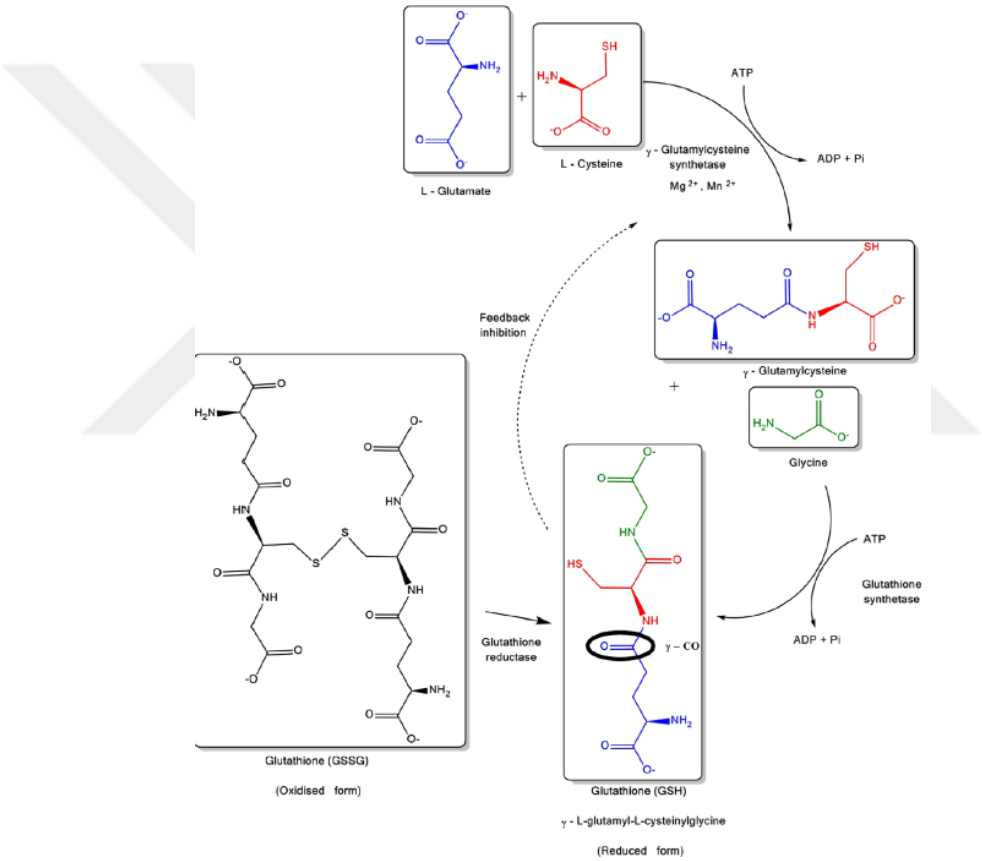

Figure 1.12. Glutathione redox cycle (39).

1.4 Environmental Monitoring Studies of Sildenafil

Emerging pollutants (EPs) are synthetic or naturally occurring substances that are not typically removed from traditional wastewater plants (42). Nevertheless, these pollutants can infiltrate ecosystems, potentially leading to acknowledged or suspected adverse impacts on both ecology and human health (42). Drugs are groups of chemicals considered as emerging pollutants. During the metabolic process, some drug molecules reach their target sites, while others convert into inactive metabolites that no longer exert effects within the body. However, many drugs are excreted either without undergoing metabolism or without being fully inactivated (43). The European Parliament established environmental quality standards and the spectrum of inorganic and organic contaminants to be monitored in water through Directive 2008/105/EC (44). The scientific community is also focusing on other pharmaceuticals that have seen a market increase in recent years, particularly PDE5 inhibitors. The use of stimulant drugs is on the rise, with concentrations in sewage treatment plants often significantly exceeding legally prescribed amounts. Phosphodiesterase type V inhibitors, such as sildenafil are among these drugs obtained illegally (45). The amount of sildenafil has been measured in several environmental monitoring studies. In a study conducted in the cities of Bristol, Brussels, Castellon, Copenhagen, Milan, Oslo, Utrecht and Zurich, it has been shown that sildenafil and its metabolites reach to the level of 60 ng/L (46). In another study conducted in Spain and German, the results have shown that sildenafil is present in 100% of all analyzed effluent water samples at levels between 6 and 18 ng/L (45). In another study conducted in UK, the concentration of sildenafil has been measured in the wastewater treatment plant influent samples at level 24.9 ng/L (47). In another study conducted in Korea, it has been shown that the concentration of PDE5 inhibitors is present in sewage influents at the level of 62 ng/L in one sample and 88 ng/L in the other and sildenafil occupy 70% of these values (48). In another study conducted in Germany, the concentration of sildenafil is present in fitness center discharges samples at level of 1.945 ng/L (49).

2. AIM AND SCOPE OF THE STUDY

Viagra is the brand name of a medication used for erectile dysfunction. The active ingredient of this medication is sildenafil citrate. Sildenafil stands out among medications that have experienced a significant surge in usage in recent years. However, the effect of this chemical on aquatic organisms has not been studied well.

Our aims in the present study were:

- To ascertain the *in vivo* impact of 2 distinct doses of sildenafil on phase I enzymes, CYP1A and CYP3A.

- To assess the *in vivo* impact of 2 distinct doses of sildenafil on Phase II enzyme, GST.

- To evaluate the *in vivo* impact of 2 distinct doses of sildenafil on the antioxidant system, encompassing CAT and GR.

3. MATERIALS AND METHODS

3.1 Materials

Sildenafil citrate ($C_{22}H_{30}N_6O_4S$, 702A021) was purchased from Solarbio. Substrates of enzyme catalyzed reactions and the chemicals of the analytical methods were the same as in our previous study and were obtained from the same company (50).

3.2 Methods

3.2.1 Animals and Treatments

3.2.1.1 Rainbow Trout Source and Experiment Place

In this study, the fish species employed is *Oncorhynchus mykiss*, commonly known as the rainbow trout. Fish treatment studies were conducted in the Laboratory of Fish of Prof. Dr. Hakan TÜRKER, BAİBÜ, Bolu, Türkiye.

3.2.1.2 Rainbow Trout Living Conditions

Fish were maintained for a period of 96 hours in 200-liter water tanks at a temperature of 10-12°C. Water quality parameters (DO, pH, TAN) in the tanks were monitored everyday with digital meters and kits. In the laboratory environment, 12h/12h light and darkness circle was applied with fluorescent lamp and timer. The tanks were continuously aerated with an aeration device (air pump and stone). The tank water and the chemical treatment were refreshed every 12 hours (Figure 3.1). Three groups were formed at varying concentrations: Group I (control group), Group II (1 µg/L sildenafil), and Group III (10 µg/L sildenafil). Each tank contained 8 fish (Figure 3.2).

Figure 3.1. Rainbow trout living conditions at laboratory. A: Different concentration of sildenafil citrate at different tanks (Control, 1 µg/L and 10 µg/L). B-C: The tank water refresh process. D: Control of water parameters.

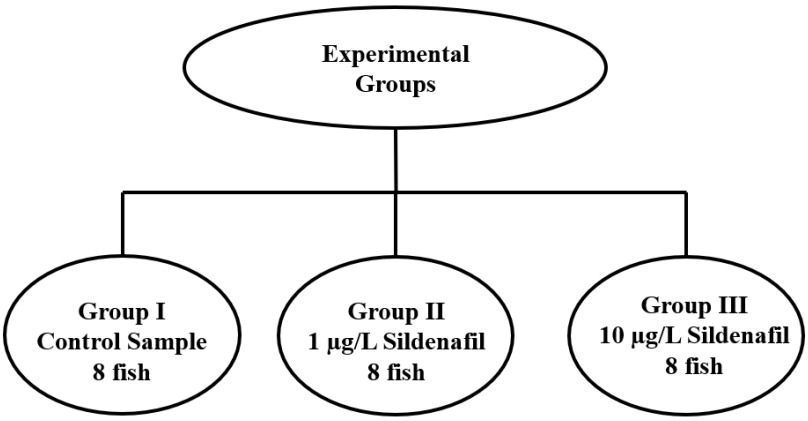

Figure 3.2. A chart depiction of groups in fish studies.

3.2.1.3 Removal of Tissues from The Animal

The experiment was terminated at the end of the 96th hour. Surgical scissors were employed to extract the tissues. Precautions were taken to prevent any contact between bile fluid and the liver tissue during the extraction process of liver. Subsequently, the tissues were labeled, packaged, and stored at -80°C ultra-low temperature freezer.

3.2.2 Tissue Extraction

The preparation of the microsome was basically conducted by the method of Arınç and Şen (1993) (51). Liver tissues retrieved from the -80°C were placed on ice. Throughout the procedure, careful attention was paid to maintenance of a cold environment for the liver tissues. The tissues were washed with cold dH_2O. After that 1.15% KCl solution were used to clean the tissues.

Liver tissues were weighted then subsequently sectioned to small fragments using surgical scissors. The minced liver samples were then homogenized in a cold solution comprising 1.15% potassium chloride, supplemented with 1.0 mM ethylenediaminetetraacetic acid at pH 7.4, 0.25 mM ε-amino caproic acid, and 0.1 mM phenylmethanesulfonyl fluoride (V=4 mL x g tissue weight). The homogenization process was conducted in ice-cooled condition utilizing a teflon pestle equipped Potter-Elvehjem glass homogenizer. The resulting homogenate was centrifuged with Sigma 3-30K Centrifuge at 10,000×g for 20 min at 4°C.

The upper phase decanted through 2 layers of cheesecloth. Subsequently, the supernatant, containing enzymes of cytoplasm and endoplasmic reticulum was subjected to centrifugation with Beckman Optima L-90K ultracentrifuge at 105,000×g for 1 hour using 70.1 Ti rotor. Following this centrifugation, the resulting pellet comprised microsomes, while the supernatant consisted of cytosol. The cytosolic fractions were stored at -80°C.

The sediment containing microsomes was resuspended in a 1.15% potassium chloride solution containing 1.0 mM ethylenediaminetetraacetic acid at pH 7.4 and subjected to ultracentrifugation at 105,000xg for 50 min to re-pelletize. Following centrifugation, the upper phase was discarded. Subsequently, precipitant part was resuspended in a 10% glycerol solution containing 1.0 mM ethylenediaminetetraacetic acid at pH 7.4 (V=1 mL x g tissue weight). The microsome samples were manually homogenized using a Potter-Elvehjem homogenizer. After that, the microsomes were stored at -80°C.

3.2.3 Quantification of Protein

Lowry et al. (1951) was utilized in the measurement of protein concentrations present in cytosols and microsomes (52). In this method, bovine serum albumin is commonly employed as a standard. The standard solutions were made ready at concentration range of 0.02-0.200 mg/mL. To each standard tubes, 0.50 mL of corresponding standard solution was put. The cytosolic proteins were quantified following centrifugation using a 12154 rotor (Sigma 3-30K Refrigerated Centrifuge, USA) at 20000 rpm for 30 min at 4°C. The upper phase was collected and utilized for the assessment of cytosolic protein content. Prior to protein quantification, microsomal and cytosolic samples were diluted 75-fold. The protein measurement procedures for both cytosolic and microsomal samples were identical. 0.25 mL or 0.5 mL aliquots of the diluted microsomal and cytosolic samples were transferred into separate tubes and the tube content was completed to 0.5 mL with dH_2O. Following this, the specimens were combined with 2.5 mL of freshly prepared alkaline copper reagent, combined from sequentially of 2.0 % sodium potassium tartrate, 2.0 % copper sulfate and 20% sodium carbonate containing 0.1 N sodium hydroxide with ratio of 1:1:100. Tubes were allowed for 10 minutes at room temperature. In the final step, the tubes were mixed with 0.25 mL of 1.0 N Folin's phenol reagent. After 30 min, absorbance values of tubes were determined at 660 nm, by using spectrophotometer (Hitachi U-2900). The standard calibration curve was drawn through the measurement of bovine serum albumin standards at various concentrations to determine the protein content of the samples.

3.2.4 Measurement of 7-EROD Activity

The method outlined by Burke and Mayer (1974) was implemented for the quantification of EROD activity in microsomes (53). The assessment of cytochrome P4501A (CYP1A) was conducted through the determination of EROD activity in microsomes. The methodology operates on the principle of converting 7-ethoxyresorufin to 7-hydroxyresorufin through the catalytic action of CYP1A with O_2 and nicotinamide adenine dinucleotide phosphate (Figure 3.3.).

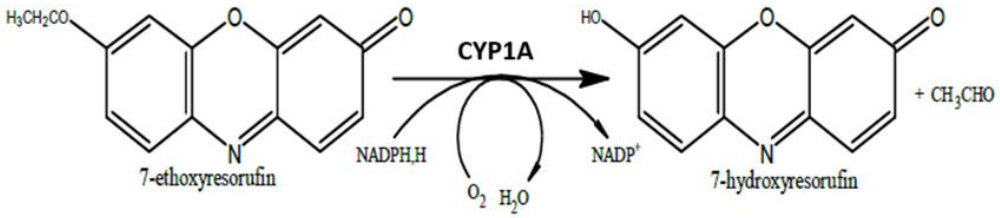

Figure 3.3. The reaction of EROD (54).

The EROD activity of each microsome was assessed under optimal conditions. Resorufin solution served as the standard, whereas 7-ethoxyresorufin solution was employed as the substrate in this protocol. Initially, stock solutions of 1.0 mM standard and 0.5 mM substrate were made ready by dissolving them with dimethyl sulfoxide. Subsequently, daily solutions of 5.0 μM resorufin and 45 μM 7-ethoxyresorufin were made ready by diluting the stock solutions with 0.2 M KPi, pH 7.8, supplemented with 0.2 M sodium chloride. The nicotinamide adenine dinucleotide phosphate generating system was prepared using 2.5 mM glucose-6-phosphate, 2.5 mM magnesium chloride, 14.6 mM N-2-hydroxyethylpiperazine-N'-2-ethansulphonic acid at pH 7.8, 0.5 U glucose-6-phosphate dehydrogenase, and 0.5 mM nicotinamide adenine dinucleotide phosphate. The solution was then kept at 37 ºC for 5 min. The reaction components were 0.1 M KPi at pH 7.8, 0.1 M sodium chloride, 1.2 mg bovine serum albumin, and 0.5 mM nicotinamide adenine dinucleotide phosphate generating system in 2.0 mL final volume. Additionally, 200 μg of microsomal protein was incorporated into spectrofluorometer cuvette. The reaction was begun by the incorporation of the nicotinamide adenine dinucleotide phosphate generating system. The progression was monitored for five min using Hitachi F-4500 spectrofluorometer at 535 nm (excitation wavelength) and 585 nm (emission wavelength). EROD activities were determined using standard calibration curves of resorufin.

3.2.5 Measurement of ERND Activity

Fish liver microsomes were utilized to ascertain the effect of sildenafil on cytochrome P4503A (CYP3A) through the enzyme activity measurement of erythromycin N-demethylase (ERND). This method was elucidated by Cochin and Axelrod (55). The method outlined by Nash was employed for quantifying the quantity of formaldehyde (56). The enzyme activity measurement depends on the formation of formaldehyde produced as a result of N-dealkylation reaction of erythromycin catalyzed by CYPA (Figure 3.4) (57).

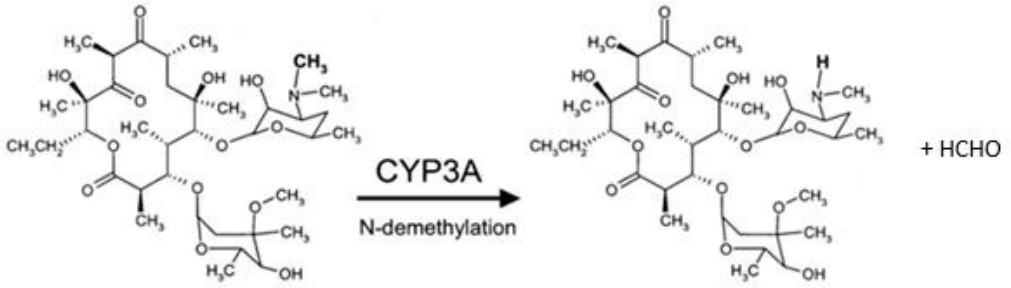

Figure 3.4. The enzymatic reaction catalyzed by erythromycin N-demethylase (57).

A standard assay solution comprised 100 mM HEPES buffer, 1 mM erythromycin (stock solution was prepared in 50% ethanol), 4.0 mg of microsomal protein and 0.5 mM nicotinamide adenine dinucleotide phosphate generating system. Blank tubes (zero time) were made ready for each sample by adding the same reaction components used in the reaction tubes. To these tubes 0.250 mL of 0.75 N perchloric acid solution was added before adding the cofactor. The reaction was initiated with the addition of nicotinamide adenine dinucleotide phosphate generating system into both the incubation mixture and the blank tubes. The reaction proceeded at a temperature of 25°C for a duration time of 30 min under agitation within a water bath. Following the waiting time, the enzymatic reaction was halted by supplementing 0.250 mL of 0.75 N perchloric acid solution to the reaction tubes. Subsequently, both the reaction mixtures and the blank tubes were subjected to centrifugation at 15000xg (Sigma 3-30K Centrifuge), for a duration of 25 min at 4°C. Fresh 0.5 mM formaldehyde stock standard solution was made ready. Standards were subsequently generated by utilizing this solution at 4 distinct concentrations: 0.012 mM, 0.024 mM, 0.050 mM, and 0.100 mM. Fresh preparation of Nash reagent was conducted by combining 0.04 mL of acetylacetone with a

solution comprising 3.08 g of ammonium acetate and 0.06 mL of glacial acetic acid in a volume of 10 mL. Following the centrifugation step, 0.250 mL were taken from both the standard and reaction tubes and transferred into test tubes. 0.188 mL of Nash reagent was added to all tubes. Then all tubes were kept in a water bath at 50°C for a duration time of 10 min. The absorbance values of the yellow coloration obtained were determined at 412 nm utilizing Hitachi U-2900 spectrophotometer. The specific activity of ERND was determined by employing the standard calibration curve of formaldehyde.

3.2.6 Measurement of Total GST Activity

The total GST activities in the cytosols of rainbow trout liver were measured in accordance with the protocol of Habig et al. (1974) (58). The methodology hinges on the enzymatic catalysis of the conjugation reaction between CDNB and GSH by GSTs, resulting in the formation of the 1-glutathione-2,4-dinitrobenzene complex (Figure 3.5) (59).

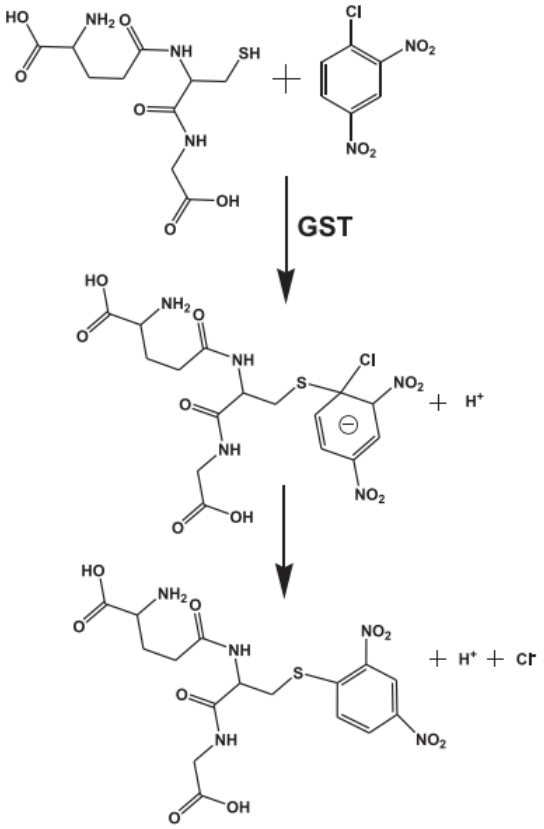

Figure 3.5. The GSH and CDNB conjugation reaction catalyzed by GST (59).

The reaction constituent in a final volume of 3.0 mL was 100 mM KPi at pH 7.4, 3.0 mM reduced glutathione, 0.150 mL cytosol (cytosols diluted were 25 times with 10 mM KPi at pH 7.4), and distilled water. The reaction commenced upon the addition of 1.0 mM CDNB to the spectrophotometer cuvette. The absorbance readings of the reaction tubes were compared to the blank tubes containing 0.150 mL KPi stock solution at pH 7.4 instead of the enzyme source. The reaction was recorded at 340 nm over a period of 2 min using Hitachi U-2900 spectrophotometer. The specific total GST activity was determined as nanomoles of 1-glutathione-2,4-dinitrobenzene conjugate formed per milligram of protein per minute at 25 ºC, employing an extinction coefficient (ε_{340}) of 0.0096 μM^{-1} cm^{-1}.

3.2.7 Measurement of CAT Activity

Catalase activities in rainbow trout liver cytosols were assessed following the protocol outlined by Aebi (1984), with some modifications (60). In this procedure, the degradation of H_2O_2 into H_2O and O_2 can be monitored in the UV range at 240 nm (Figure 3.6).

$$2\,H_2O_2 \xrightarrow{\text{Catalase}} 2\,H_2O + O_2$$

Figure 3.6. Catalase Reaction (60).

Prior to the catalase enzyme activity measurement, pretreatment of rainbow trout cytosol was done with 1% Triton X-100 (1:10) for 10 minutes. Subsequently, 25-fold further dilution was done with 50 mM phosphate buffer at pH 7.0. The sample was diluted 250 times in total. The reaction was begun by the addition of 1.0 mL of 150 mM H_2O_2 in 50 mM KPi solution to the cuvette containing 2.0 mL of diluted diluted cytosol. This mixture represents a standard reaction mixture used to measure the catalase activity present in rainbow trout liver cytosol.

The absorbance readings of the reaction tubes were compared with the blank tubes containing 1.0 mL of 50 mM KPi at pH 7.0 instead of H_2O_2. The reaction was recorded at 240 nm for a duration of 1 min using spectrophotometer (Hitachi U-2900). The specific catalase activity was determined as micromoles of H_2O_2 consumed per milligram of protein per minute at 25 ºC, employing an extinction coefficient (ε_{240}) of 0.0364 mM^{-1} cm^{-1}.

3.2.8 Measurement of GR Activity

The methodology introduced by Carlberg and Mannervick (1985) was employed for assessing the activity of glutathione reductase in liver cytosol (61). GR catalyzes the reduction of oxidized glutathione to its reduced form using nicotinamide adenine dinucleotide phosphate. According to the equation, one molecule of nicotinamide adenine dinucleotide phosphate is utilized to reduce one molecule of oxidized glutathione (Figure 3.7).

Figure 3.7. GSH formation from decomposition of GSSG with nicotinamide adenine dinucleotide phosphate (40).

In the glutathione reductase activity assay, the reaction medium consisted of 0.5 mM ethylenediaminetetraacetic acid at pH 7.0, 100 mM KPi at pH 7.0, 0.1 mM nicotinamide adenine dinucleotide phosphate, 50 µL of enzyme source, and dH_2O in 2.0 mL final volume. To initiate the reduction reaction, 1.0 mM glutathione disulfide was introduced into the spectrophotometer cuvette. The utilization of NADPH was quantified spectrophotometrically (Hitachi U-2900) at 340 nm over a period of 5 min. Enzyme activities of the samples were calculated using an extinction coefficient (ε_{340}) of 6.22 mM^{-1} cm^{-1}.

3.3 Statistical Analysis

Data were analyzed with SPSS Statistics 21.0 program. First of all, data were analyzed with Shapiro–Wilk test for normality. Parametric data were analyzed by LSD test. Mann-Whitney U-test was used for nonparametric data. Results were given as mean enzyme activity ± SEM in tables.

4. RESULTS

Samples of rainbow trout were collected from a nearby rainbow trout fish farm located in Bolu. The rainbow trout specimens were divided into 3 distinct groups. The group I served as the control, while the group II and III were exposed to sildenafil at concentrations of 1 µg/L and 10 µg/L, respectively. Following the treatment, liver samples were extracted from the rainbow trout specimens. Microsomal and cytosolic fractions were isolated from the liver samples of rainbow trout. Enzyme activity measurements were done in these fractions.

4.1 EROD Enzyme Activity

The assessment of cytochrome P4501A-associated EROD activity was conducted with each microsome samples. The results were presented in Tables 4.1-4.3. The mean EROD activity of the control group was 14.73 ± 1.89 pmole/min/mg protein. The mean EROD activity of rainbow trout in the group treated with 1 µg/L sildenafil was 23.65 ± 4.96 pmole/min/mg protein. The mean EROD activity of rainbow trout in the group treated with 10 µg/L sildenafil was 13.43 ± 1.30 pmole/min/mg protein. The EROD activity results were shown in Figure 4.1. No statistically significant difference was found when the activities of the treatment groups were compared with the control group.

Table 4.1. EROD activities of the control group.

Sample Number	Average Activity (pmol/min/mg protein)
1	20.44
2	15.16
3	22.38
4	13.06
5	6.99
6	9.96
7	18.26
8	11.58
Average Activity±SEM N=8	14.73 ± 1.89

Table 4.2. EROD activities of the group treated with 1 µg/L sildenafil.

Sample Number	Average Activity (pmol/min/mg protein)
1	27.32
2	50.42
3	11.78
4	6.99
5	17.11
6	14.31
7	31.29
8	29.98
Average Activity±SEM N=8	23.65 ± 4.96

Table 4.3. EROD activities of the group treated with 10 µg/L sildenafil.

Sample Number	Average Activity (pmol/min/mg protein)
1	21.57
2	12.45
3	14.17
4	10.08
5	14.50
6	10.47
7	13.08
8	11.15
Average Activity±SEM N=8	13.43 ± 1.30

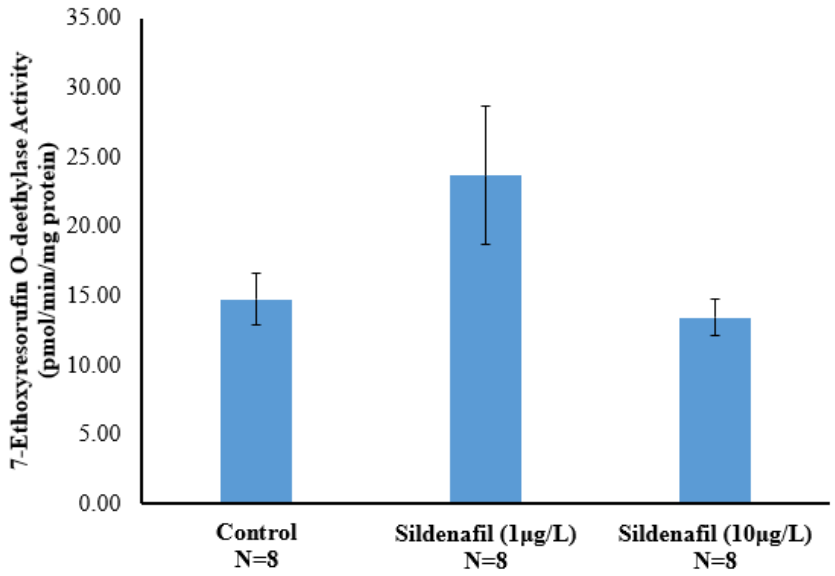

Figure 4.1. EROD activities of all groups.

4.2 ERND Enzyme Activity

The ERND activities were assessed in the liver microsomes. The results were showed in Tables 4.4-4.6. The mean ERND activity of the control group was 0.030 ± 0.016 pmole/min/mg protein. The mean ERND activity of the group treated with 1 µg/L sildenafil was 0.054 ± 0.018 pmole/min/mg protein. The mean ERND activity of the group treated with 10 µg/L sildenafil was 0.015 ± 0.006 pmole/min/mg protein. ERND activities were shown together in Figure 4.2. No statistically significant difference was found between the groups.

Table 4.4. ERND activities of the control group.

Sample Number	Average Activity (pmol/min/mg protein)
1	0.010
2	0.026
3	0.000
4	0.104
5	0.104
6	0.000
7	0.000
8	0.000
Average Activity±SEM N=8	0.030 ± 0.016

Table 4.5. ERND activities of the group treated with 1 µg/L sildenafil.

Sample Number	Average Activity (pmol/min/mg protein)
1	0.126
2	0.118
3	0.052
4	0.089
5	0.051
6	0.000
7	0.000
8	0.000
Average Activity±SEM N=8	0.054 ± 0.018

Table 4.6. ERND activities of the group treated with 10 µg/L sildenafil.

Sample Number	Average Activity (pmol/min/mg protein)
1	0.000
2	0.048
3	0.033
4	0.017
5	0.019
6	0.000
7	0.000
8	0.000
Average Activity±SEM N=8	0.015 ± 0.006

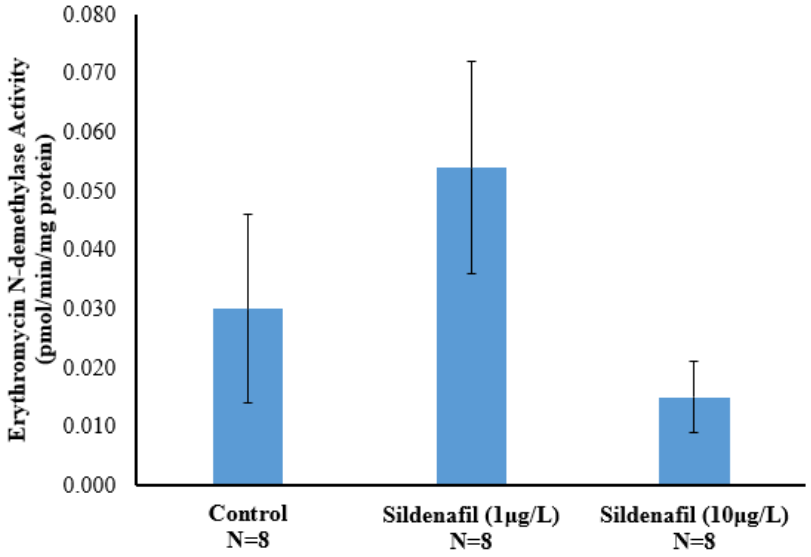

Figure 4.2. ERND activities of all groups.

4.3 Total GST Enzyme Activity

The cytosolic total GST activities were quantified in the liver of rainbow trout. The results were presented in Tables 4.7-4.9. The mean total GST activity of rainbow trout in the control group was 936 ± 40 nmol/min/mg protein. The mean total GST activity of the group treated with 1 µg/L sildenafil was 810 ± 56 nmol/min/mg protein. The mean total GST activity of the group treated with 10 µg/L sildenafil was 781 ± 80 nmol/min/mg protein. GST activities were shown together in Figure 4.3. No statistically significant difference was found between the groups.

Table 4.7. GST activities of the control group.

Sample Number	Average Activity (nmol/min/mg protein)
1	836
2	995
3	1054
4	1089
5	802
6	880
7	1007
8	828
Average Activity±SEM N=8	936 ± 40

Table 4.8. GST activities of the group treated with 1 µg/L sildenafil.

Sample Number	Average Activity (nmol/min/mg protein)
1	579
2	697
3	912
4	754
5	1005
6	652
7	918
8	964
Average Activity±SEM N=8	810 ± 56

Table 4.9. GST activities of the group treated with 10 µg/L sildenafil.

Sample Number	Average Activity (nmol/min/mg protein)
1	561
2	1034
3	809
4	1029
5	814
6	498
7	530
8	976
Average Activity±SEM N=8	781 ± 80

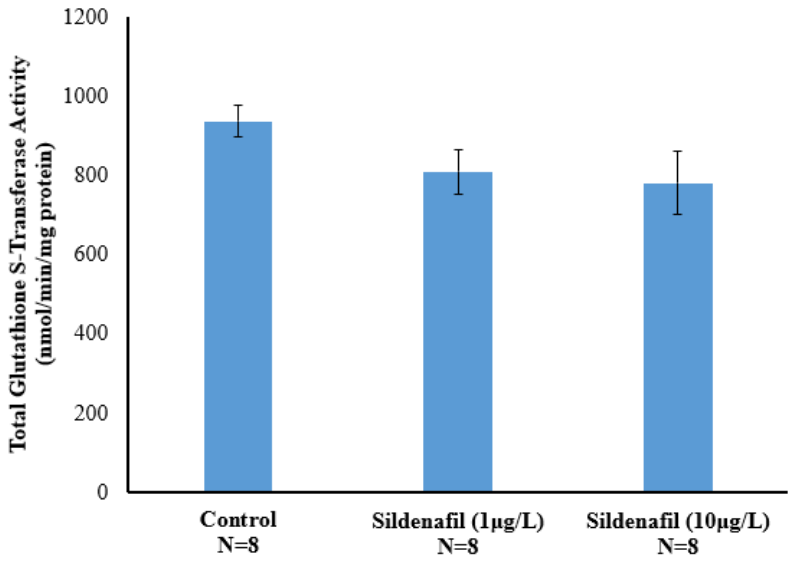

Figure 4.3. Total GST activities of all groups.

4.4 CAT Enzyme Activity

The cytosolic CAT activities were quantified in the liver of rainbow trout. The results were presented in Tables 4.10-4.12. The mean CAT activity of the control group was 192 ± 20 µmol/min/mg protein. The mean CAT activity of the group treated with 1 µg/L sildenafil was 232 ± 13 µmol/min/mg protein. The mean CAT activity of the group treated with 10 µg/L sildenafil was 204 ± 29 µmol/min/mg protein. CAT activities were shown together in Figure 4.4. No statistically significant difference was found between the groups.

Table 4.10. CAT activities of the control group.

Sample Number	Average Activity (µmol/min/mg protein)
1	136
2	218
3	154
4	316
5	187
6	144
7	186
8	198
Average Activity±SEM N=8	192 ± 20

Table 4.11. CAT activities of the group treated with 1 µg/L sildenafil.

Sample Number	Average Activity (µmol/min/mg protein)
1	226
2	168
3	236
4	245
5	205
6	289
7	266
8	218
Average Activity±SEM N=8	232 ± 13

Table 4.12. CAT activities of the group treated with 10 µg/L sildenafil.

Sample Number	Average Activity (µmol/min/mg protein)
1	234
2	309
3	126
4	60
5	190
6	186
7	230
8	296
Average Activity±SEM N=8	204 ± 29

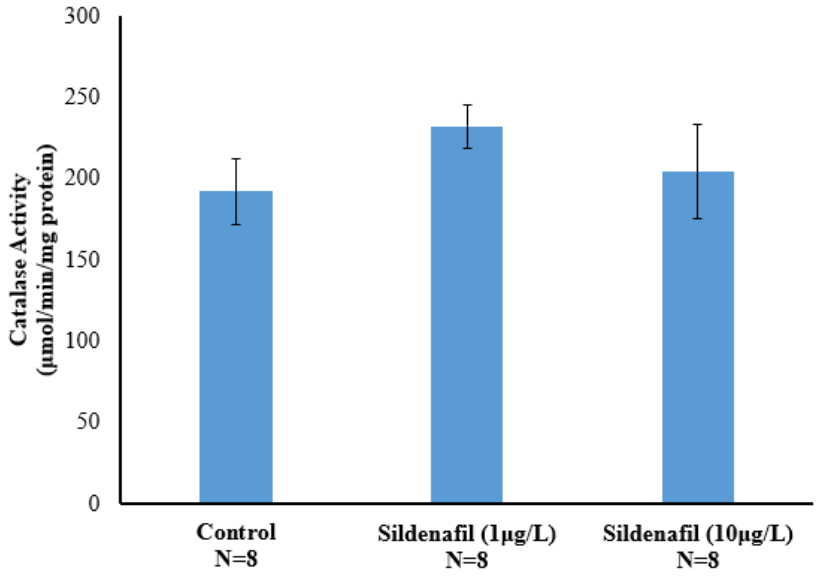

Figure 4.4. CAT activities of all groups.

4.5 GR Enzyme Activity

The cytosolic GR activities were quantified in the liver of rainbow trout. The results were presented in Tables 4.13-4.15. The mean GR activity of the control group was 37.27 ± 4.77 nmol/min/mg protein. The mean GR activity of the group treated with 1 µg/L sildenafil was 27.79 ± 3.16 nmol/min/mg protein. The mean GR activity of the group treated with 10 µg/L sildenafil was 27.01 ± 2.06 nmol/min/mg protein. GR activities were shown together in Figure 4.5. No statistically significant difference was found between the groups.

Table 4.13. GR activities of the control group.

Sample Number	Average Activity (nmol/min/mg protein)
1	27.66
2	47.02
3	59.64
4	47.45
5	24.06
6	41.62
7	26.93
8	23.80
Average Activity±SEM N=8	37.27 ± 4.77

Table 4.14. GR activities of the group treated with 1 µg/L sildenafil.

Sample Number	Average Activity (nmol/min/mg protein)
1	18.66
2	20.63
3	46.66
4	21.17
5	32.55
6	26.67
7	27.62
8	28.38
Average Activity±SEM N=8	27.79 ± 3.16

Table 4.15. GR activities of the group treated with 10 µg/L sildenafil.

Sample Number	Average Activity (nmol/min/mg protein)
1	24.46
2	32.87
3	33.74
4	27.00
5	27.38
6	20.08
7	18.31
8	32.23
Average Activity±SEM N=8	27.01 ± 2.06

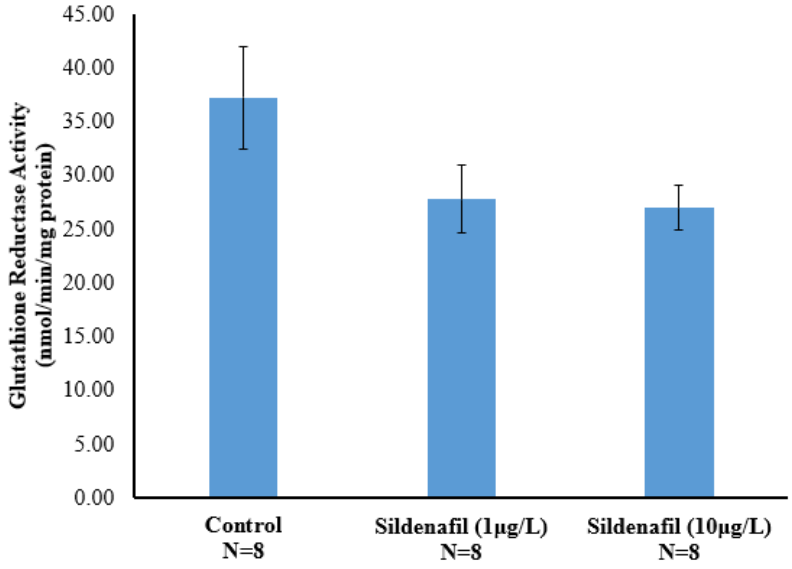

Figure 4.5. GR activities of all groups.

5. DISCUSSION

Sildenafil (Viagra) is among the drugs that is used to treat erectile dysfunction in men (6, 7, 9, 62). Sildenafil is not normally found in natural water sources. It is transferred to these systems via sewage activities. The information about the effect of this chemical on fish is limited. The studies show that some of the drug molecules used by human have negative effects on the fish endocrine system (63-65). The number of fish decreases day by day. Overfishing is an important factor in this decrease in fish numbers. However, the adverse effects of the chemicals on the reproduction, metabolism and growth of fish may also be the other reasons related with this decrease.

Drug molecules used by humans can have inhibitory or activating effects on various enzyme activities. The same enzymes have role in the metabolism of other chemicals to which the fish are exposed. Sildenafil is a specific inhibitor of cGMP-specific PDE5 (9). cGMP is a substance that causes blood vessels (arteries) to relax and widen. PDE5 works by breaking down cyclic guanosine monophosphate. Sildenafil increases the duration of action of cGMP by inhibiting the PDE5 enzyme. This causes the veins to relax or widen. As a result, erection duration increases (6-9).

Recently, the use of sildenafil has increased world-wide. In a recent *in vivo* study conducted on rats, it has been observed that sildenafil increases hepatocyte cGMP level (66). However, several studies have reported that sildenafil causes hepatotoxic effects in human and laboratory animals (67-69). In a study it has been found that sildenafil administration in wistar rats causes alterations in liver functions. Additionally, hepatotoxic effect has not been completely reversed stopping sildenafil administration to rats (68). In a different study, rats fed with ethanol were administered sildenafil to examine the effects of the drug in conjunction with alcohol consumption. Sildenafil has been found to exacerbate liver damage when it is consumed with ethanol. It is concluded that the consumption of phosphodiesterase 5 inhibitors, such as sildenafil, in combination with alcohol is not advisable for liver health (69). On the other hand, its effect has not been well studied in fish. It has been demonstrated that genetic effects and apoptotic cell formation increases after 21- and 28-days sildenafil exposure period in zebra fish (70). In another study, antioxidant enzyme activities increase in caged juveniles of

Prochilodus lineatus expose to sewage effluents containing caffeine, atenolol, carbamazepine, enalapril and sildenafil (71). However, fish samples expose to mixture of drug molecules in this study.

The purpose of this study is to determine the impact of sildenafil on cytochrome P450 system and antioxidant enzyme activities. Cytochrome P450 system and antioxidant enzyme activities are involved in the metabolism of endogenous and exogenous (xenobiotic substances in organism) molecules (20, 21, 50). Therefore, changes as a result of sildenafil exposure in fish may not only provide information about the effect of this chemical, but also may produce information about how the metabolism of other molecules are affected by this chemical.

Rainbow Trout (*Oncorhynchus mykiss*) is a very popular fish fed in Türkiye and all around the world and scientist have used them for investigation. In this study, the impact of sildenafil was measured in the liver of fish treated with two different doses of sildenafil for 96 hours. The research was designed to determine the acute effect of sildenafil in fish. Sildenafil is rapidly absorbed and distributed molecule. It has been found that sildenafil administered orally is absorbed very quickly, reaching its maximum level in the blood in approximately 60 minutes (67). Metabolomics studies have shown us that sildenafil has 12 phase I metabolites in humans (13).

Drug molecules are metabolized with two phase reactions. These are phase I and phase II reactions. The majority of phase I reactions in the endoplasmic reticulum are catalyzed by cytochrome P450 enzymes. CYPs catalyze their reactions in the presence of cytochrome P450 reductase enzyme. The CYP enzymes are enzymes containing heme groups. CYPs are involved in the metabolism of endogenous and exogenous substances (20, 21, 50, 72). It has been shown that sildenafil is mainly metabolized by CYPs (13, 14, 67).

Cytochrome P4501A (CYP1A), one of the cytochrome P450 enzymes, is involved in the metabolic reactions of many organic chemicals, PAHs, PCBs and dioxins. In the presence of these chemicals, its amount increases, and this increase can be monitored both at the enzyme activity level and at the protein amount level. Cytochrome P4501A enzyme level is determined by spectrofluorometric determination of EROD activity (23, 24). There is a *in vivo* study conducted on male rats treated with sildenafil. According to this study, cytochrome P450 enzymes

are affected from sildenafil. Moreover, they have observed reduction in protein expression and significant inhibition in EROD activity (73). In the current study, the effect of sildenafil citrate on CYP1A activity was determined by measuring EROD activities in microsomes prepared from liver tissues. EROD activities of the group treated with 1 μg/L sildenafil was higher than the control and the group treated with 10 μg/L sildenafil. However, no statistically significant difference was found when the EROD activities of sildenafil treated groups were compared with the control group. This result clearly showed that CYP1A protein was not significantly affected from the doses of sildenafil citrate used in this study.

Cytochrome P4503A is the other cytochrome P450 enzyme measured in this study. This enzyme generally participates detoxification type of reactions. In general, it involves in the metabolism of most of the drugs. Its activity is generally followed by erythromycin N-demethylase (ERND) activity (74). In a study, the metabolism of sildenafil has been examined using recombinant human CYP enzymes and human liver microsomes, and the role of CYP3A has been shown in sildenafil metabolism in human (75). ERND activities of the group treated with 1 μg/L sildenafil was higher than the control and the group treated with 10 μg/L sildenafil. However, no statistically significant difference was found when the ERND activities of sildenafil treated groups were compared with the control group. This result clearly showed that CYP3A protein was not significantly affected from the doses of sildenafil citrate used in this study.

Drugs that undergo biotransformation through Phase I reactions are then converted into less fat-soluble by Phase II (conjugation) reactions and are eliminated from the body. Some chemicals carry groups suitable for conjugation reactions in their structures. These chemicals can be eliminated from the body directly by Phase II reactions. GST is an enzyme involved in Phase II reactions and carries out the conjugation reaction in the presence of glutathione (27-29). The cytosolic glutathione S-transferase and enzymes involved in glutathione metabolism are essential for detoxifying numerous endogenous and exogenous substances (27-29, 76). In a study it has been reported that GST activity is not affected from the sildenafil treatment in male rats (76). In another study carried out in the liver of *Prochilodus lineatus*, GST activity increases in fish exposed to water polluted with drugs including sildenafil (77). However, these fish samples expose to other chemicals since polluted water may contain many other chemicals. In this

study, GST activities of the groups treated with 1 μg/L and 10 μg/L sildenafil were lower than the control group. However, no statistically significant difference was found when the GST activities of sildenafil treated groups were compared with the control group. This result clearly showed that GST activity was not significantly affected from the doses of sildenafil citrate used in this study.

Metabolism of drug molecules may also generate ROS and modify antioxidant system. GR and glutathione have crucial role in the elimination of free radicals (38, 76). In a study, it has been reported that GR activity decreases in sildenafil treated male rats (76). Moreover, sildenafil treatment increases catalase activity in the liver tissue of rats in the same study. In another study examining the effect of sildenafil on the antioxidant system, it has been observed that sildenafil increases catalase activity and inhibits GR activity in the liver of rabbits (78). In this study, GR activities of the groups treated with 1 μg/L and 10 μg/L sildenafil were lower than the control group. CAT activities of the group treated with 1 μg/L sildenafil were higher than the control group. However, no statistically significant differences were found when the GR and CAT activities of sildenafil treated groups were compared with the control group. This result clearly showed that GR and CAT activities were not significantly affected from the doses of sildenafil citrate used in this study.

6. CONCLUSIONS AND RECOMMENDATIONS

Sildenafil is predominantly used in the treatment of erectile dysfunction. With the increase in its use, it has begun to be measured in various aquatic environments. Recently, aquatic organisms are under the risk of sildenafil exposure. In this research, our purpose was to determine the impact of 2 different doses of sildenafil on CYP1A-associated EROD, CYP3A-associated ERND, CAT, GST and GR activities in rainbow trout. There was no significant difference between control and sildenafil treatment groups in all activities measured in this research. It is clear that sildenafil did not modify any of these activities at the doses of sildenafil citrate used in this study.

In this study, the effect of sildenafil was determined within short period of time. The effect of sildenafil may be seen when the treatment period and the amount of sildenafil are increased. The chronic study can be done with higher doses of sildenafil.

7. REFERENCES

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